Enzymes from a Thermophilic bacterium, *Pyrococcus furiosus*

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The discovery of hyperthermophilic archaebacteria has provided a valuable tool for the analysis of protein stability. The intrinsic thermal stability of the enzymes isolated from these sources makes it possible to study the molecular mechanisms governing structure and function in a system adapted for elevated temperatures. The thermostability exhibited by these enzymes is maintained without any components unique to thermophiles, suggesting that the increase in molecular stability is accomplished through the same stereochemical interactions found in their mesophilic counterparts. The characteristic range of activity observed in hyperthermophilic enzymes tends to parallel growth temperature, there being little or no activity at temperatures which would be optimal for their mesophilic counterparts. Through analysis of these enzymes it should be possible to determine the stabilizing interactions by which the enzymes maintain activity at extreme temperatures.

Pyrococcus furiosus is an anaerobic marine heterotroph with an optimal growth temperature of 100°C, isolated by Fiala and Stetter (1986) from solfataric mud off the coast of Vulcano island, Italy. The α-amylase from *Pyrococcus furiosus* has been purified to homogeneity. The enzyme is a homodimer with a subunit molecular mass of 66 kDa. The isoelectric point is 4.3. The enzyme displays optimal activity, with substantial thermal stability, at 100°C, with the onset of activity at approximately 40°C. Unlike mesophilic α-amylases there is no dependence on Ca^{2+} for activity or thermostability. The enzyme displays a broad range of substrate specificity, with the capacity to hydrolyze carbohydrates as simple as maltotriose. No substrate binding occurs below the

temperature threshold of activity, and a decrease in K_m accompanies an increase in temperature. UV spectroscopy and intrinsic fluorescence measurements detected no temperature-dependent structural reorganization. Hydrogen exchange results indicate that the molecule is rigid, with only a slight increase in conformational flexibility at elevated temperature. Scanning microcalorimetry detected no considerable change in the heat capacity function, at the pH of optimal activity, within the temperature range in which activity is induced. The heat absorption peak due to denaturation, under these conditions, occurred within the temperature range of 90-120°C. At temperatures below 90°C no excess heat absorption or change in the CD spectra were observed which could be associated with the cooperative conformational transition of the protein.

Our current investigations involve the isolation and characterization of two additional enzymes from sonicates of *Pyrococcus furiosus*, DNase and a serine proteinase. These enzymes also exhibit maximum activity at temperatures in the neighborhood of 100°C. Following physical characterization of these proteins as a function of temperature, we plan to study the tertiary structure of one of the three proteins by x-ray crystallography.